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ART UNIT	**PAPER NUMBER**
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/611,629	GUO, BAOCHUAN	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-27 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-27 is/are rejected.

7)☒ Claim(s) 1 is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 01 July 2003 is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date 1/19/04
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Priority

1. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed application, Provisional Application No. 60/392,251, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. With regard to claim 21, the detection of mutant NR-21 microsatellites is not disclosed. Accordingly, claim 21 has not been granted benefit of the earlier filing date, and the original filing date, July 1, 2003, has been used for prior art purposes.

Specification

2. The disclosure is objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded

hyperlink and/or other form of browser-executable code. See MPEP § 608.01. Specifically, page 21 of the specification recites http://www-genome.wi.mit.edu/cgi--bin/primer/primer3_www.cgi.. Removal of the "http" would satisfy this requirement.

Claim Objections

3. Claim 1 is objected to because of the following informalities: a typographical error causes step (e) to be listed twice. Appropriate correction is required.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 25-27 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

6. The term "limiting" in claim 25, step (c) is a relative term that renders the claim indefinite. The term "limiting" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Specifically, it is unclear to what extent extension of a primer annealed to a wild type polynucleotide is "limited" by annealing of the probe.

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 1-2, 5-6, 14-15, 18, 25 and 27 are rejected under 35 U.S.C. 102(a) as being anticipated by Sun et al. (Nature Biotechnology, February 2002).

With regard to claim 1, Sun et al. disclose a method for detecting a mutant polynucleotide in a mixture of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides, comprising the steps of:

a) selecting an extension primer complementary to a first target sequence in the mutant and wild-type polynucleotides (Experimental protocol, page 188, where in the second round of PCR primers to the constant region are complementary to both wild-type and mutant polynucleotides)

b) selecting a probe complementary to a second target sequence in the wild-type polynucleotides but not in the mutant polynucleotides, wherein the second target sequence is located 3' of the first target sequence on the same polynucleotide strand (Experimental protocol, page 188, where the PNA probe hybridizes to the wild-type but not the mutant sequence)

c) contacting the polynucleotides in the mixture with the probe under conditions where the probe preferentially anneals to the second target sequence rather than to a corresponding sequence in the mutant polynucleotides (Experimental protocol, pages 188-189, specifically the second round of PCR where the PNA probe is included)

d) contacting the polynucleotides in the mixture with the extension primer under conditions where the primer anneals to the first target sequence (Experimental protocol, pages 188-189, specifically the second round of PCR where the PNA probe is included with primers complementary to the constant region)

e) contacting the polynucleotides in the mixture with a DNA polymerase and nucleoside triphosphates under conditions where the extension primers are extended by polynucleotide synthesis, using the mutant and wild-type polynucleotides as templates, to produce extension products, wherein polynucleotide synthesis that uses the wild-type polynucleotides as templates is blocked by the probe (Experimental protocol, pages 188-189)

e) isolating the extension products from the mixture (Experimental protocol, page 189, where ZipTips are used to isolate extension products)

f) amplifying the extension products produced from mutant polynucleotide templates preferentially over amplifying extension products produced from wild-type polynucleotide templates using the polymerase chain reaction (PCR) (page 189, the second round of PCR including the PNA probe amplifies the extension products).

With regard to claim 2, Sun et al. disclose the method of claim 1, wherein the mutant polynucleotides contain substitution mutations, as compared to the wild-type polynucleotides (page 187, column 1, first paragraph).

With regard to claim 5, Sun et al. disclose the method of claim 1, wherein the extension primer has one or more attached biotin molecules (page 189 where the K-ras primer is biotinylated at the 5'end).

With regard to claim 6, Sun et al. disclose the method of claim 1, wherein the probe is a peptide nucleic acid (Experimental protocol, page 188).

With regard to claim 14, Sun et al. disclose the method of claim 1, wherein polynucleotide synthesis preferentially extends extension primers that have annealed to the first target sequence in mutant polynucleotides (Experimental protocol, pages 188-189, where the use of the PNA probe results in preferential extension of the primers annealed to the first target sequence in mutant polynucleotides).

With regard to claim 15, Sun et al. disclose the method of claim 1, wherein the extension products are isolated from the mixture by a solid phase extraction method (page 189, where isolation using chromatographic ZipTips is a solid phase extraction method).

With regard to claim 18, Sun et al. disclose the method of claim 1, comprising the additional step of analyzing the amplified extension products from the PCR (page 189, where the extension products are analyzed by MALDI-TOF).

With regard to claim 25, Sun et al. disclose a method for selectively amplifying a mutant polynucleotide, if any, in a mixture of wild-type polynucleotides and unrelated

polynucleotides, both the mutant polynucleotide and the wild-type polynucleotides having the same first target sequence, the wild-type polynucleotide having a second target sequence not present in the mutant polynucleotide, at least part of the second target sequence being located 3' of the first target sequence on the same polynucleotide strand of the wild-type polynucleotide, the process comprising:

a) contacting the mixture with a probe complementary to the second target sequence to preferentially anneal the probe to the second target sequence of the wild-type polynucleotide rather than to a corresponding sequence in the mutant polynucleotide (Experimental protocol, pages 188-189, where the PNA probe hybridizes to the wild-type but not the mutant sequence)

b) contacting the mixture with an extension primer complementary to the first target sequence in both the mutant and wild-type polynucleotides to anneal the primer to the first target sequence in both polynucleotides (Experimental protocol, page 188, where in the second round of PCR primers to the constant region are complementary to both wild-type and mutant polynucleotides)

c) contacting the mixture with a DNA polymerase and nucleoside triphosphates to extend the extension primers annealed to the polynucleotides by polynucleotide synthesis, the probe annealed to the wild-type polynucleotide limiting polynucleotide synthesis of the extension primer annealed to the wild-type polynucleotide (Experimental protocol, pages 188-189).

With regard to claim 27, Sun et al. disclose the process of claim 25, further comprising determining the size and abundance of amplified extended extension

primers from the PCR (page 189, where MALDI-TOF provides an measure of the size and abundance of the amplified extension products).

9. Claims 1-2, 7-12, 14, 18, 25, and 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Rampersad et al. (US Patent No. 5,830,712).

With regard to claim 1, Rampersad et al. disclose a method for detecting a mutant polynucleotide in a mixture of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides, comprising the steps of:

a) selecting an extension primer complementary to a first target sequence in the mutant and wild-type polynucleotides (Example 2, column 6, lines 25-30, where the degenerate oligonucleotides are complementary to wild-type and mutant polynucleotides. Also note that Rampersad et al. state that the claimed method is designed to inactivate undesirable members, where undesirable members are defined as those members of the sample that interfere with the use of the sample. The disclosure of Rampersad et al. encompasses undesirable members that are wild-type or mutant polynucleotides)

b) selecting a probe complementary to a second target sequence in the wild-type polynucleotides but not in the mutant polynucleotides, wherein the second target sequence is located 3' of the first target sequence on the same polynucleotide strand (Example 2, column 6, lines 33-40, where the "blockers" satisfy these limitations)

c) contacting the polynucleotides in the mixture with the probe under conditions where the probe preferentially anneals to the second target sequence rather than to a

corresponding sequence in the mutant polynucleotides (Example 2, column 6, lines 41-45)

d) contacting the polynucleotides in the mixture with the extension primer under conditions where the primer anneals to the first target sequence (Example 2, column 6, lines 41-45)

e) contacting the polynucleotides in the mixture with a DNA polymerase and nucleoside triphosphates under conditions where the extension primers are extended by polynucleotide synthesis, using the mutant and wild-type polynucleotides as templates, to produce extension products, wherein polynucleotide synthesis that uses the wild-type polynucleotides as templates is blocked by the probe (Example 2, column 6, lines 41-67)

e) isolating the extension products from the mixture (Example 2, column 6, lines 41-67 and abstract where the extension products may be isolated)

f) amplifying the extension products produced from mutant polynucleotide templates preferentially over amplifying extension products produced from wild-type polynucleotide templates using the polymerase chain reaction (PCR) (Example 2, column 6, lines 41-67, where the extended products are produced via PCR).

With regard to claim 2, Rampersad et al. disclose the method of claim 1, wherein the mutant polynucleotides contain deletion mutations, insertion mutations, substitution mutations or a combination of deletion, insertion and substitution mutations, as compared to the wild-type polynucleotides (see abstract and column 2, lines 44-50

where the nucleic acid family inherently includes deletions, insertions, substitutions, and combinations thereof).

With regard to claim 7, Rampersad et al. disclose the method of claim 1, wherein the probe is an oligonucleotide (Example 2, column 6, lines 33-37).

With regard to claim 8, Rampersad et al. disclose the method of claim 7, wherein at least part of the oligonucleotide has a phosphorothioated backbone (column 3, lines 65-67).

With regard to claim 9, Rampersad et al. disclose the method of claim 7, wherein the oligonucleotide has a 5' end and a 3' end and the 3' end is modified such that it cannot be extended by polynucleotide synthesis (column 3, lines 52-53).

With regard to claim 10, Rampersad et al. disclose the method of claim 9, wherein the nucleotide at the 3' end of the oligonucleotide is phosphorylated (column 3, lines 63-64).

With regard to claim 11, Rampersad et al. disclose the method of claim 1, wherein the probe is a modified oligonucleotide (column 3, lines 63-67; where the addition of an amine, phosphate, acridine or cholesterol group to the probe results in a modified oligonucleotide).

With regard to claim 12, Rampersad et al. disclose the method of claim 1, wherein:

a) there is a first T_m for annealing of the extension primer to the first target sequence (column 6, lines 46-53, where the first T_m is 52°C)

b) there is a second T_m for annealing of the probe to the second target sequence (column 6, lines 46-53, where the second T_m is 65°C)

c) there is a third T_m for annealing of the probe to the mutant polynucleotides, wherein the second T_m is higher than the first T_m; and wherein the first T_m is higher than the third T_m (see Example 2, lines 46-67, note that T_{m2} (65°C) > T_{m1} (52°C)).

With regard to step (c) above, although Rampersad et al. do not specifically disclose the presence of a third T_m that is lower than the first T_m, this is inherently implied. Rampersad et al. explicitly discuss the importance of conducting the PCR under conditions where the blocker oligonucleotide can only hybridize to the undesirable sequences, and particularly point out that the T_m of the blocker oligonucleotide must be higher than the T_m of the primers (column 6, lines 49-61). Furthermore, for the method of Rampersad et al. to function properly, the T_m for probe hybridization to the undesirable sequences (analogous to the instant wild-type sequences), must be lower than the T_m for primer annealing. Otherwise, the probe would hybridize to the desirable and undesirable sequences, thereby preventing preferential amplification of the desirable sequences, which is the object of the method of Rampersad et al. Therefore, the method of Rampersad et al. must include a third T_m , for hybridization of the blocker oligonucleotide to the mutant (desirable) seqeucnes, and this T_m must be lower than the T_m for primer annealing. Thus, the disclosure of Rampersad et al. meets the instant limitations.

With regard to claim 14, Rampersad et al. disclose the method of claim 1, wherein polynucleotide synthesis preferentially extends extension primers that have

annealed to the first target sequence in mutant polynucleotides (Example 2, column 6, lines 25-67, where practice of the method results in preferential extension of primers annealed to desirable sequences and extension is blocked by the hybridization of the blocker oligonucleotide to the undesirable sequences).

With regard to claim 18, Rampersad et al. disclose the method of claim 1, comprising the additional step of analyzing the amplified extension products from the PCR (column 5, lines 63-65 and Figure 1, where the amplified products were analyzed by agarose gel electrophoresis).

With regard to claim 25, Rampersad et al. disclose a method for selectively amplifying a mutant polynucleotide, if any, in a mixture of wild-type polynucleotides and unrelated polynucleotides, both the mutant polynucleotide and the wild-type polynucleotides having the same first target sequence, the wild-type polynucleotide having a second target sequence not present in the mutant polynucleotide, at least part of the second target sequence being located 3' of the first target sequence on the same polynucleotide strand of the wild-type polynucleotide, the process comprising:

a) contacting the mixture with a probe complementary to the second target sequence to preferentially anneal the probe to the second target sequence of the wild-type polynucleotide rather than to a corresponding sequence in the mutant polynucleotide (Example 2, column 6, lines 33-45, where the "blockers" satisfy these limitations)

b) contacting the mixture with an extension primer complementary to the first target sequence in both the mutant and wild-type polynucleotides to anneal the primer

to the first target sequence in both polynucleotides (Example 2, column 6, lines 25-30 and 41-45, where the degenerate oligonucleotides are complementary to wild-type and mutant polynucleotides. Also note that Rampersad et al. state that the claimed method is designed to inactivate undesirable members, where undesirable members are defined as those members of the sample that interfere with the use of the sample. The disclosure of Rampersad et al. encompasses undesirable members that are wild type or mutant polynucleotides.)

c) contacting the mixture with a DNA polymerase and nucleoside triphosphates to extend the extension primers annealed to the polynucleotides by polynucleotide synthesis, the probe annealed to the wild-type polynucleotide limiting polynucleotide synthesis of the extension primer annealed to the wild-type polynucleotide (Example 2, column 6, lines 41-67).

With regard to claim 27, Rampersad et al. disclose the process of claim 25, further comprising determining the size and abundance of amplified extended extension primers from the PCR (column 5, lines 63-65 and Figure 1, where agarose gel electrophoresis provides a measure of the size and abundance of the amplified extension products).

10. Claims 1-3, 6, 12-14, 17, 18, 25 and 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Orum et al. (Nucleic Acids Research, 1993).

With regard to claim 1, Orum et al. disclose a method for detecting a mutant polynucleotide in a mixture of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides, comprising the steps of:

a) selecting an extension primer complementary to a first target sequence in the mutant and wild-type polynucleotides (page 5335, Figure 6, where the common reverse primer is complementary to both the wild-type and mutant polynucleotides)

b) selecting a probe complementary to a second target sequence in the wild-type polynucleotides but not in the mutant polynucleotides, wherein the second target sequence is located 3' of the first target sequence on the same polynucleotide strand (page 5335, Figure 6, where the PNA62 probe hybridizes to the wild-type, but not the mutant plasmid)

c) contacting the polynucleotides in the mixture with the probe under conditions where the probe preferentially anneals to the second target sequence rather than to a corresponding sequence in the mutant polynucleotides (page 5335, Figure 6)

d) contacting the polynucleotides in the mixture with the extension primer under conditions where the primer anneals to the first target sequence (page 5335, Figure 6)

e) contacting the polynucleotides in the mixture with a DNA polymerase and nucleoside triphosphates under conditions where the extension primers are extended by polynucleotide synthesis, using the mutant and wild-type polynucleotides as templates, to produce extension products, wherein polynucleotide synthesis that uses the wild-type polynucleotides as templates is blocked by the probe (Figure 6, page 5335)

e) isolating the extension products from the mixture (Figure 6, page 5335, where the products are isolated by agarose gel electrophoresis)

f) amplifying the extension products produced from mutant polynucleotide templates preferentially over amplifying extension products produced from wild-type polynucleotide templates using the polymerase chain reaction (PCR) (Figure 6, page 5335, where the extension products are produced by PCR).

With regard to claim 2, Orum et al. disclose the method of claim 1, wherein the mutant polynucleotides contain substitution mutations, as compared to the wild-type polynucleotides (Figure 6, page 5335, where the mutant plasmids contain point substitutions).

With regard to claim 3, Orum et al. disclose the method of claim 1, wherein the mutant and wild-type polynucleotides are isolated from the mixture of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides before the step of contacting the polynucleotides with the probe (Methods section, page 5333, first and third paragraphs, where the wild-type and mutant plasmids were separately isolated from a mini-library and then added to the PCR mixture at concentrations of 0.1 ng per plasmid, thereby eliminating the presence of unrelated polynucleotides).

With regard to claim 6, Orum et al. disclose the method of claim 1, wherein the probe is a peptide nucleic acid (Figure 6, page 5335, where the PNA62 probe is a peptide nucleic acid).

With regard to claim 12, Orum et al. disclose the method of claim 1, wherein:

a) there is a first T_m for annealing of the extension primer to the first target sequence (Figure 6, where the primers anneal at 40°C)

b) there is a second T_m for annealing of the probe to the second target sequence (Figure 6, where the probe anneals at 65°C)

c) there is a third T_m for annealing of the probe to the mutant polynucleotides, wherein the second T_m is higher than the first T_m (65°C > 40°C) and wherein the first T_m is higher than the third T_m (page 5334, column 2 – page 5335 column 1 and Figure 5, where Orum et al. state that when the second T_m is higher than the first T_m (61C versus 52C), amplification of both the wild-type and mutant sequences is inhibited (Figure 5, lane 2). Orum et al. further state that upon increasing the size of the mutant primer (and thereby increasing its T_m above the T_m of the probe this effect could be eliminated (Figure 5, lanes 8 and 10). Therefore, the disclosure of Orum et al. meets the instant limitations.

With regard to claim 13, Orum et al. disclose the method of claim 1, wherein the first target sequence and the second target sequence overlap (pages 5333-5334 teach the testing of overlapping first and second sequences and the results shown in Figure 6 were obtained using the "primer exclusion" configuration, where the sites overlap (page 5334, column 2).

With regard to claim 14, Orum et al. disclose the method of claim 1, wherein polynucleotide synthesis preferentially extends extension primers that have annealed to the first target sequence in mutant polynucleotides (Figure 6, page 5335, where practice of the method results in preferential extension of primers annealed to the first target

sequences in mutant plasmids sequences and extension is blocked by the hybridization of the PNA probe to the wild type sequences).

With regard to claim 17, Orum et al. disclose the method of claim 1, wherein the PCR uses:

a) a first PCR primer that is complementary to a nucleotide sequence present in the 3' end of a long extension product, but not present in a short extension product (Figure 6, page 5335, where the allele-specific primer for the mutant sequences is complementary to a sequence present at the 3' end of a long extension product (the amplified mutant sequence), and is not present in a short (wild type) extension product)

b) a second PCR primer that is identical to a nucleotide sequence present in both the long and short extension products (Figure 6, page 5335, where the common reverse primer is identical to a sequence present in both wild type and mutant products).

With regard to claim 18, Orum et al. disclose the method of claim 1, comprising the additional step of analyzing the amplified extension products from the PCR (Figure 6, page 5335, where the extension products are analyzed by agarose gel electrophoreisis).

With regard to claim 25, Orum et al. disclose a method for selectively amplifying a mutant polynucleotide, if any, in a mixture of wild-type polynucleotides and unrelated polynucleotides, both the mutant polynucleotide and the wild-type polynucleotides having the same first target sequence, the wild-type polynucleotide having a second target sequence not present in the mutant polynucleotide, at least part of the second

target sequence being located 3' of the first target sequence on the same polynucleotide strand of the wild-type polynucleotide, the process comprising:

a) contacting the mixture with a probe complementary to the second target sequence to preferentially anneal the probe to the second target sequence of the wild-type polynucleotide rather than to a corresponding sequence in the mutant polynucleotide (Figure 6, page 3555, where the PNA62 probe meets these limitations)

b) contacting the mixture with an extension primer complementary to the first target sequence in both the mutant and wild-type polynucleotides to anneal the primer to the first target sequence in both polynucleotides (page 3555, Figure 6, where the common reverse primer meets these limitations)

c) contacting the mixture with a DNA polymerase and nucleoside triphosphates to extend the extension primers annealed to the polynucleotides by polynucleotide synthesis, the probe annealed to the wild-type polynucleotide limiting polynucleotide synthesis of the extension primer annealed to the wild-type polynucleotide (page 3555, Figure 6).

With regard to claim 27, Orum et al. disclose the process of claim 25, further comprising determining the size and abundance of amplified extended extension primers from the PCR (Figure 6, page 3555, where the size and abundance is determined by agarose gel electrophoresis).

11. Claims 1-2, 7, 9, 11, 14, 18 and 25-27 are rejected under 35 U.S.C. 102(b) as being anticipated by Seyama et al. (Nucleic Acids Research, 1992).

With regard to claim 1, Seyama et al. disclose a method for detecting a mutant polynucleotide in a mixture of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides, comprising the steps of:

a) selecting an extension primer complementary to a first target sequence in the mutant and wild-type polynucleotides (page 2494, Methods section and Figure 1, where the PN12-1 and PN12-2 primers are complementary to wild type and mutant N-*ras* sequqences)

b) selecting a probe complementary to a second target sequence in the wild-type polynucleotides but not in the mutant polynucleotides, wherein the second target sequence is located 3' of the first target sequence on the same polynucleotide strand (page 2494, Methods section and Figure 1, where the blockers BN12-1 and BN12-2 are complementary to a wild-type N-ras sequence located 3' of the first target sequence on the same strand)

c) contacting the polynucleotides in the mixture with the probe under conditions where the probe preferentially anneals to the second target sequence rather than to a corresponding sequence in the mutant polynucleotides (Figure 1 and Methods section, first paragraph, page 2494)

d) contacting the polynucleotides in the mixture with the extension primer under conditions where the primer anneals to the first target sequence (Figure 1 and Methods section, first paragraph, page 2494)

e) contacting the polynucleotides in the mixture with a DNA polymerase and nucleoside triphosphates under conditions where the extension primers are extended by

polynucleotide synthesis, using the mutant and wild-type polynucleotides as templates, to produce extension products, wherein polynucleotide synthesis that uses the wild-type polynucleotides as templates is blocked by the probe (Figure 1 and Methods section, first paragraph, page 2494)

e) isolating the extension products from the mixture (Methods section, first paragraph, page 2494, where the extension products are isolated by agarose gel electrophoresis)

f) amplifying the extension products produced from mutant polynucleotide templates preferentially over amplifying extension products produced from wild-type polynucleotide templates using the polymerase chain reaction (PCR) (Methods section, first paragraph, page 2494, where the extension products are produced by PCR).

With regard to claim 2, Seyama et al. disclose the method of claim 1, wherein the mutant polynucleotides contain substitution mutations, as compared to the wild-type polynucleotides (page 2494, column 2, where the tumor cell lines used contain base change mutations TGT and GAT at codon 12 of the N-ras gene (normally GGT)).

With regard to claim 7, Seyama et al. disclose the method of claim 1, wherein the probe is an oligonucleotide (Methods section, page 2494).

With regard to claim 9, Seyama et al. disclose the method of claim 7, wherein the oligonucleotide has a 5' end and a 3' end and the 3' end is modified such that it cannot be extended by polynucleotide synthesis (Methods section, page 2494, where the blockers contain a 3'terminal dideoxynucleotide to prevent chain elongation).

With regard to claim 11, Seyama et al. disclose the method of claim 1, wherein the probe is a locked nucleic acid, modified oligonucleotide or oligonucleotide analogue (Methods section, page 2494, where the addition of the dideoxynucleotide to the 3'terminus of the blocker results in a "modified oligonucleotide").

With regard to claim 13, Seyama et al. disclose the method of claim 1, wherein the first target sequence and the second target sequence overlap.

With regard to claim 14, Seyama et al. disclose the method of claim 1, wherein polynucleotide synthesis preferentially extends extension primers that have annealed to the first target sequence in mutant polynucleotides (page 2493, column 2, last paragraph).

With regard to claim 18, Seyama et al. disclose the method of claim 1, comprising the additional step of analyzing the amplified extension products from the PCR (Methods section, page 2494, where the extension products are analyzed by agarose gel electrophoresis and single strand conformation polymorphism (SSCP) analysis).

With regard to claim 25, Seyama et al. disclose a method for selectively amplifying a mutant polynucleotide, if any, in a mixture of wild-type polynucleotides and unrelated polynucleotides, both the mutant polynucleotide and the wild-type polynucleotides having the same first target sequence, the wild-type polynucleotide having a second target sequence not present in the mutant polynucleotide, at least part of the second target sequence being located 3' of the first target sequence on the same polynucleotide strand of the wild-type polynucleotide, the process comprising:

a) contacting the mixture with a probe complementary to the second target sequence to preferentially anneal the probe to the second target sequence of the wild-type polynucleotide rather than to a corresponding sequence in the mutant polynucleotide (Figure 1 and Methods section, page 2494)

b) contacting the mixture with an extension primer complementary to the first target sequence in both the mutant and wild-type polynucleotides to anneal the primer to the first target sequence in both polynucleotides (Figure 1 and Methods section, page 2494)

c) contacting the mixture with a DNA polymerase and nucleoside triphosphates to extend the extension primers annealed to the polynucleotides by polynucleotide synthesis, the probe annealed to the wild-type polynucleotide limiting polynucleotide synthesis of the extension primer annealed to the wild-type polynucleotide (Methods section, first paragraph, page 2494).

With regard to claim 26, Seyama et al. disclose the process of claim 25, further comprising:

a) isolating extended extension primers from the mixture (Methods section, page 2494 and Figure 3B)

b) contacting the extended extension primers with a first and second PCR primer, a PCR polymerase and nucleoside triphosphates to amplify the extended extension primers by PCR, the PCR preferentially amplifying extended extension primers from extension primers annealed to mutant polynucleotides (Methods section and Figure 3B, where the products shown in Figure 3A are subjected to a second PCR amplification).

With regard to claim 27, Seyama et al. disclose the process of claim 25, further comprising determining the size and abundance of amplified extended extension primers from the PCR (Methods section, page 2494, where agarose gel electrophoresis provides a measure of the size and abundance of the amplified extension products).

Claim Rejections - 35 USC § 103

12. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

13. Claims 3, 4 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over either Seyama et al. (Nucleic Acids Research, 1992), Rampersad et al. (US Patent No, 5,830,712) or Sun et al. (Nature Biotechnology, 2002) in view of Nollau et al. (Clinical Chemistry and Laboratory Medicine, 1999).

Seyama et al., Rampersad et al. or Sun et al. teach the method of claim 1, as discussed above.

With regard to claim 3, none of the above references (Seyama et al., Rampersad et al., Sun et al.) teach that the mutant and wild type polynucleotides are isolated from unrelated polynucleotides before the step of contacting the polynucleotides with the probe.

With regard to claim 4, none of the above references (Seyama et al., Rampersad et al., Sun et al.) teach the use of a sequence specific hybrid capture method to isolate the mutant and wild type polynucleotides from the unrelated polynucleotides.

With regard to claim 16, none of the above references (Seyama et al., Rampersad et al., Sun et al.) teach preferential isolation of mutant polynucleotides.

Nollau et al. teach a method for enrichment of mutant alleles by chromatographic removal of wild type alleles. Single-stranded PCR products "were loaded onto a chromatographic column containing streptavidin sepharose to which biotinylated oligonucleotides were bound that were complementary to the wild type sequence. The wild-type sequences bound to their immobilized complements and the flow-through contained an enriched population of mutant alleles. (See Methods section, and the first paragraph of Results, page 878).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to use a sequence specific capture method as taught by Nollau et al. in order to remove unrelated sequences prior to the amplification of mutant alleles using the methods taught by any of Seyama et al., Rampersad et al. or Sun et al. Nollau et al. taught that the chromatographic capture method described above produced a population highly enriched in mutant sequences (abstract: "enrichment of one mutant in up to 1,000 normal alleles has been achieved"). Where an initial purification step to remove unrelated sequences is desired prior to performing the blocker-PCR method of either Seyama et al., Rampersad et al. or Sun et al., the teachings of Nollau et al. would have provided strong motivation to employ the aforementioned sequence specific

capture method. Two simple modifications would have been required of the ordinary artisan: (1) addition of a biotin moiety to the common reverse primers of Seyama et al., Rampersad et al. or Sun et al., and (2) immobilization of sequences complementary to wild type and mutant sequences in the column of Nollau et al. These modifications would have been easily effected and were clearly within the level of ordinary skill.

With regard to claim 16, following enrichment by the method taught by either of Seyama et al., Rampersad et al. or Sun et al., it would have been obvious to the person of ordinary skill, given the teachings of Nollau et al., to include a further purification/enrichment step comprising removal of any contaminating wild-type sequences using the aforementioned chromatographic separation method. Such a method, when performed as taught by Nollau et al., would have eliminated any residual wild type contaminants, thereby producing a further purified (preferentially isolated) enriched population of mutant sequences. As discussed above, the biotinylation and immobilization of wild-type specific primers would have been easily accomplished by the person of ordinary skill. Therefore, one of ordinary skill, interested in obtaining a highly purified, maximally enriched population of mutant polynucleotides, would have been motivated to incoroporate the chromatographic separation method of Nollau et al. in the methods of either Seyama et al., Rampersad et al. or Sun et al. to remove unrelated polynucleotides and/or preferentially isolate mutant sequences, thus resulting in the instantly claimed methods.

14. Claims 19-20, 22, and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shuber (US Patent No. 6,280,947 B1) in view of Rampersad et al. (US Patent No. 5,830,712) or Sun et al. (Nature Biotechnology, Feb. 2002).

Rampersad et al. and Sun et al. separately teach the method of claim 1, as discussed above.

With regard to claim 19, the method disclosed by Rampersad et al. or Sun et al. discussed above meets the limitations of the instant claim with the exception that instant method uses a microsatellite templates.

With regard to claim 23, Rampersad et al. teach the use of multiplexing, but not with regard to microsatellites (column 4, lines 19-22, where the use of multiple blockers is taught). Sun et al. also teach multiplexing (pages 188-189, where K-ras and p53 primers are used), but also not with regard to microsatellites.

Shuber teaches a method of detecting mutations comprising primer extension with labeled nucleotides (column 3, lines 25-47).

With regard to claim 20, Shuber teaches that the microsatellites are BAT26 microsatellites (column 5, lines 18-31).

With regard to claim 22, Shuber teaches the use of genomic DNA from a stool or blood sample (column 4, lines 8-10).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to use the method of mutation detection taught by Sun et al. or Rampersad et al. to improve the sensitivity of the microsatellite mutation assay taught by Shuber. Shuber taught that deletions in the BAT-26 locus were a useful marker for

colorectal cancer and taught a primer extension assay using nucleic acids isolated from stool and labeled nucleotides for their detection. Shuber further taught that these nucleic acid targets might only comprise only about 1-5% of the sample, thereby necessitating an amplification step, such as PCR (column 4, lines 13-17). The mutant allele enrichment methods taught by Sun et al. and Rampersad et al. would have been directly applicable to the ordinary practitioner of the method of Shuber, because incorporation of a mutant allele enrichment step would have greatly increased the level of mutant polynucleotides in the sample, thereby providing increased sensitivity in the subsequent primer extension assay of Shuber. Futhermore, Sun et al. and Rampersad et al. separately taught that the mutant allele enrichment method could be multiplexed to detect multiple targets, thereby motivating the ordinary practitioner of the method of Shuber to utilize a multiplexed assay to simultaneously detect the BAT-26 and APC 1309 mutations, which were detected separately in Examples 1 and 2 of Shuber (columns 9-12). Therefore, the person of ordinary skill, interested in increasing the sensitivity and streamlining the microsatellite detection method of Shuber, would have been motivated to incorporate the mutant allele enrichment methods separately taught by Sun et al. and Rampersad et al., thus resulting in the instantly claimed methods.

15. Claims 21 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shuber (US Patent No. 6,280,947 B1) in view of Rampersad et al. (US Patent No. 5,830,712) or Sun et al. (Nature Biotechnology, 2002) and further in view of Percesepe

et al. (Genes Chromosomes Cancer, 2000) and Suraweera et al. (Gastroenterology, Dec. 2002).

The combined teachings of Rampersad et al. or Sun et al. with the teachings of Shuber result in the method of claim 19, as discussed above.

Neither Rampersad et al., Sun et al., or Shuber teach the detection of the NR-21 microsatellite or the simultaneous detection of the BAT-26 and TGF-B RII microsatellites.

With regard to claim 21, Suraweera et al. teach that NR-21 microsatellites are associated with colon cancer (page 1809, column 1, last paragraph).

With regard to claim 24, Percesepe et al. teach that BAT-26 and TGF- B RII microsatellites are associated with colon cancer (abstract).

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to detect mutations in the NR-21 microsatellites and/or simultaneously detect mutations in the TGF- B RII and BAT-26 microsatellites when performing the combined methods of Shuber and Rampersad et al. or Sun et al. The method of Shuber is directed in some embodiments to the detection of microsatellite mutations indicative of colon cancer (column 5, lines 18-31). Percesepe et al. and Suraweera et al. taught that mutations in the NR-21, BAT-26 and TGF- B RII microsatellites were useful colon cancer markers (abstract of Percesepe et al. and page 1809, column 1 of Suraweera et al.). These teachings of Percesepe et al. and Suraweera et al. would have motivated the ordinary practitioner of the method of Shuber to include these microsatellites in order to provide additional positive indicators of cancer and improve

the accuracy of the resulting diagnosis. As discussed above, Rampersad et al. and Sun et al. separately disclosed multiplexing, so simultaneous detection of BAT-26 and TGF-B RII would have been well within the capabilities of the ordinary artisan. Therefore, one of ordinary skill in the art, interested in obtaining a more accurate diagnosis of colon cancer using the method of Shuber modified to include the mutant allele enrichment method of Rampersad et al. or Sun et al. would have been motivated to further include the NR-21, BAT-26 and TGF-B RII microsatellites, thus resulting in the instantly claimed methods.

Conclusion

16. No claims are currently allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is (571) 272-8291. The examiner can normally be reached on M-F 7:30-5 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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2/24/06